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STIMULATION OF BREATHING MOVEMENTS BY L-5-HYDROXYTRYPTOPHAN IN FETAL SHEEP DURING NORMOXIA AND HYPOXIA

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SUMMARY

1. In fetal lambs in late gestation, systemic infusion of L-5-hydroxytryptophan (L-5-HTP) during normoxia greatly increases the incidence of fetal breathing movements (FBM) and high-voltage electrocortical activity (HV ECoG). It also induces FBM during HV ECoG and increases blood pressure. To investigate its mechanism of action, L-5-HTP was administered in conjunction with the 5-hydroxytryptamine (5-HT) antagonists ketanserin or cyproheptadine. L-5-HTP was also infused with or without the antagonists during hypoxia, to test whether it would overcome the inhibition of FBM by hypoxia.

2. When L-5-HTP was given in normoxia, cyproheptadine blocked and ketanserin reduced the increase in blood pressure, both drugs blocked the stimulation of FBM, but neither drug prevented the induction of prolonged episodes of HV ECoG.

3. In hypoxia, L-5-HTP similarly stimulated FBM. This effect was also blocked by cyproheptadine and was delayed by ketanserin.

4. The antagonism of the effects of L-5-HTP on blood pressure and the incidence of FBM in normoxia and hypoxia is consistent with the action of L-5-HTP via 5-HT receptors. At present there is no clear explanation of the mechanism by which L-5-HTP induces HV ECoG.

INTRODUCTION

The amino acid L-5-hydroxytryptophan (L-5-HTP) is converted by L-aromatic amino acid decarboxylase, which is widely distributed within the central nervous system, to the neurotransmitter 5-hydroxytryptamine (5-HT; Cooper, Bloom & Roth, 1982). While 5-HT does not cross the blood–brain barrier (Axelrod & Inscoe, 1963), L-5-HTP is readily transported and hence is a simple means of increasing 5-HT levels in the central nervous system.

Post-natally, 5-HT is implicated in the control of the cardiovascular and respiratory systems and of behaviour. Both facilitatory and inhibitory effects have

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been reported on respiration (Dempsey, Olson & Skatrud, 1986), and similarly complex effects have been observed on blood pressure (Goodman Gilman, Goodman, Rall & Murad, 1985). More consistent effects are seen on behaviour, where 5-HT is considered an important hypnogenic factor (see Jouvet, 1972, and Koella, 1984, for reviews). Thus in many species systemic administration of the 5-HT precursor L-5-HTP, or injection of 5-HT within the brain, induces slow-wave sleep. Peak levels of 5-HT and its metabolites coincide with sleep periods, whereas reduction of 5-HT levels by parachlorophenylalanine or 5-methoxy dimethyl tryptamine, or by lesions in the principal 5-HT-containing areas of the brain, induce insomnia. Restoration of 5-HT levels by L-5-HTP reverses the insomnia and the restoration is prevented by 5-HT receptor antagonists.

5-Hydroxytryptamine may be an important neurotransmitter in the control of both breathing and behaviour in the fetus. Quilligan, Clewlow, Johnston & Walker (1981) showed that intravenous infusion of L-5-HTP greatly increased the incidence of fetal breathing movements (FBM) and high-voltage electrocortical activity (HV ECoG) and it even induced FBM during HV ECoG. The effects of L-5-HTP on FBM are important because one of the principal differences between breathing pre- and post-natally is that, in fetal sheep in late gestation, FBM do not occur either continuously or at random but in discrete episodes, confined to periods of low-voltage electrocortical activity (LV ECoG) (Dawes, Fox, Leduc, Liggins & Richards, 1972). The means whereby this pattern changes to continuous breathing at birth is not known. We therefore wished to investigate the mechanism of action of L-5-HTP and have examined whether its effects are blocked by the 5-HT antagonists ketanserin or cyproheptadine (Leysen, Awouters, Kennis, Laduron, Vandenberk & Janssen, 1981).

A second important difference between breathing pre- and post-natally is in the response to hypoxia; FBM are inhibited by isocapnic hypoxia (Boddy, Dawes Fisher, Pinter & Robinson, 1974; Clewlow, Dawes, Johnston & Walker, 1983), and this seems appropriate to survival *in utero* as the oxygen supply to the fetus depends on maternal breathing. The means whereby this response changes to a stimulation of breathing by hypoxia after birth is not known. It has been proposed that the inhibition of FBM by hypoxia is mediated by a descending inhibitory pathway, since after transection of the fetal brain stem at the level of the colliculi (Dawes, Gardner, Johnston & Walker, 1983), or bilateral pontine lesions in the region of the trigeminal nuclei (Gluckman & Johnston, 1987), breathing is increased in amplitude and frequency by hypoxia. One approach to understanding this inhibition is to examine whether neurotransmitter agonists or antagonists will restore FBM during hypoxia. We have therefore investigated whether infusion of L-5-HTP into fetal lambs during isocapnic hypoxia will cause FBM to reappear and whether this is also blocked by the two 5-hydroxytryptamine receptor antagonists.

Some of these results have already been presented as a preliminary communication (Hanson, Moore, Nijhuis & Parkes, 1987).

METHODS

Chronically instrumented fetal sheep were prepared as described previously (Hanson, Moore, Nijhuis & Parkes, 1988). Briefly, nine cross-bred ewes were anaesthetized at 109–120 days gestation (term is *ca.* 144 days) with halothane (1·5–2% in O_2; Dawes *et al.* 1972). Electrodes were implanted in the fetus to measure diaphragm electromyographic and electrocortical activity and catheters were placed for measurement of pressure in a fetal carotid artery, the trachea and the amniotic cavity (Dawes *et al.* 1972; Clewlow *et al.* 1983). A catheter in the fetal jugular vein was used to infuse drugs. Five days were allowed for post-operative recovery, during which time antibiotics were administered to the ewe and the fetus.

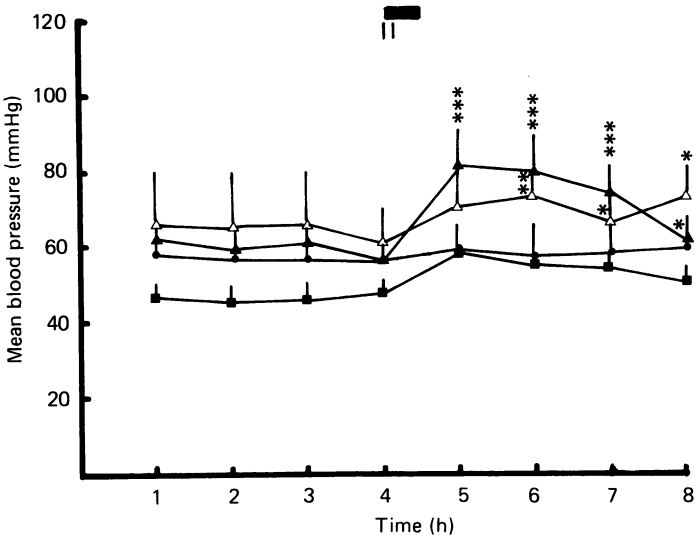

Fig. 1. The effects on mean (+S.E.M.) blood pressure over the 8 h period. ▬ indicates when saline or L-5-HTP was infused and || indicates injection of saline, ketanserin or cyproheptadine. ●, ▲, ■, △ indicate the experiments with saline (six animals), L-5-HTP (six animals), L-5-HTP with cyproheptadine (five animals) or L-5-HTP with ketanserin (five animals). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ *vs.* saline. After infusion, all differences between L-5-HTP and L-5-HTP with cyproheptadine were significant and during hours 5 and 6 the differences between L-5-HTP and L-5-HTP with ketanserin were significant (not indicated).

Experimental protocol

Experiments in normoxia. Previous pilot experiments confirmed the doses of ketanserin and cyproheptadine that appeared to block completely the effects of L-5-HTP. These are similar to those which antagonize 5-HT-induced behavioural and cardiovascular responses post-natally (e.g. Stone, Wanger, Ludden, Stavorski & Ross, 1961; Awouters, 1985; Goodman Gilman *et al.* 1985). L-5-Hydroxytryptophan (120 mg, Sigma) was dissolved in saline and infused into the fetal jugular vein over 35 min. Ketanserin (6 mg, Janssen) or cyproheptadine (3 mg, Sigma) were dissolved in saline and injected into the fetal carotid artery 5 min before and again 10 min after the start of the infusion of L-5-HTP. The same protocol was followed in all animals substituting saline for either L-5-HTP or the antagonists, so that each animal could serve as its own saline-infused control. The sequence of experiments on different days was varied between animals and only one experiment per animal was performed each day. During the 4 h before and the 4 h after the start of the infusion the number of minutes per hour of rapid irregular FBM of at least 1 min duration, HV ECoG and FBM during HV ECoG were separately measured. Measurements of mean blood pressure were made hourly over the same 8 h period.

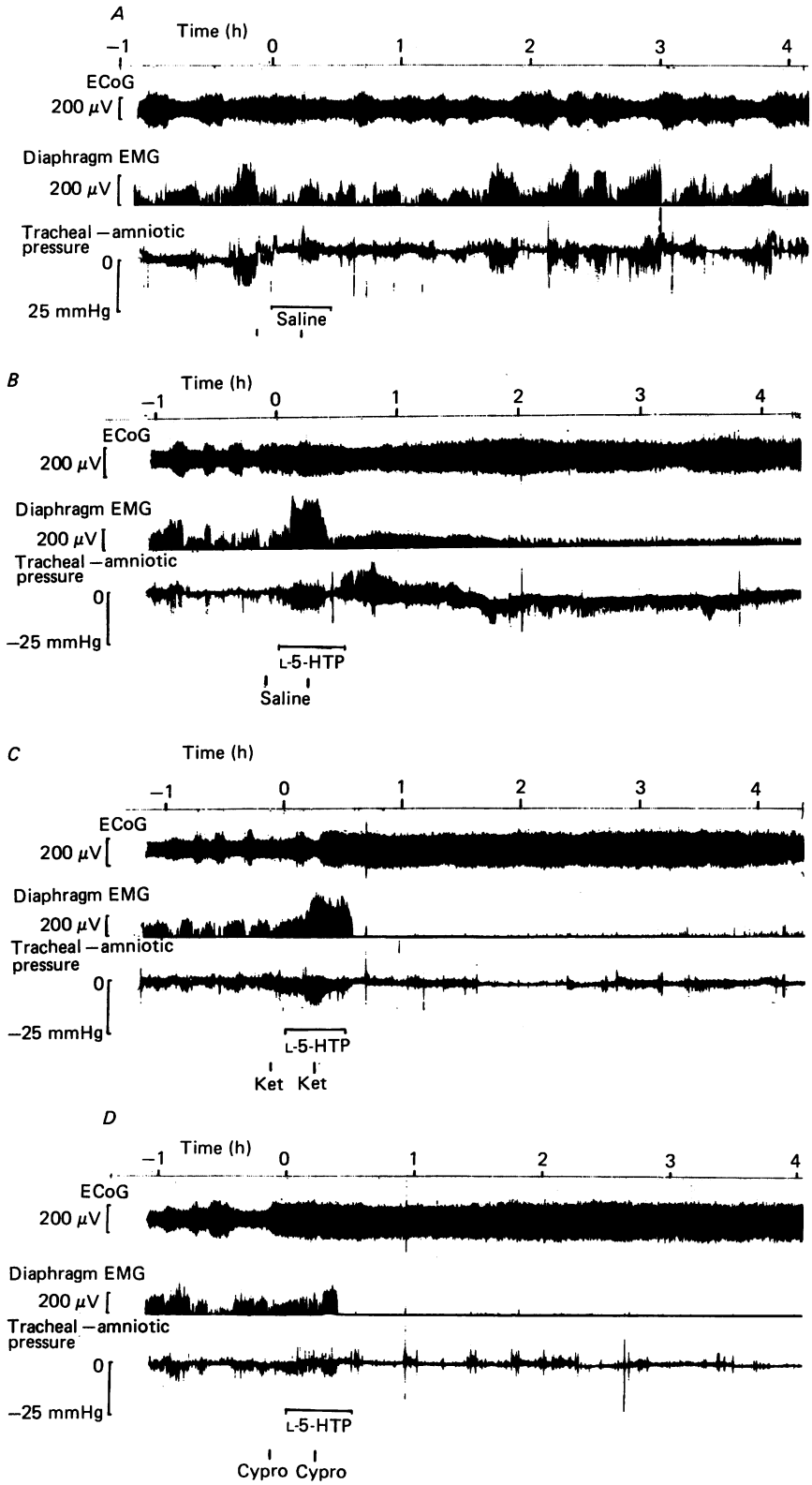

Fig. 2. For legend see opposite.

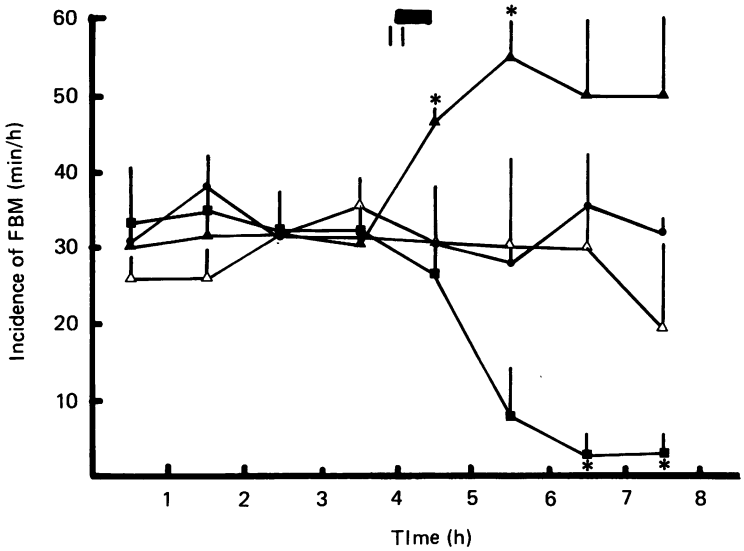

Fig. 3. The effects on mean (+S.E.M.) incidence of FBM over the 8 h period. ▬ indicates when saline or L-5-HTP was infused and || indicates injection of saline, ketanserin or cyproheptadine. ●, ▲, ■, △ indicate the experiments with saline (seven animals), L-5-HTP (seven animals), L-5-HTP with cyproheptadine (six animals) or L-5-HTP with ketanserin (six animals). *$P < 0.05$ *vs.* saline. After infusion, all differences between L-5-HTP and L-5-HTP with cyproheptadine or L-5-HTP and L-5-HTP with ketanserin were significant (not indicated).

Experiments in hypoxia. Isocapnic hypoxia was induced by administration to the ewe of an inspirate consisting of 9% O_2 and 3% CO_2 in N_2. After 20 min the fetus was infused intravenously with saline or L-5-HTP (120 mg). Ketanserin, (6 mg), cyproheptadine (3 mg) or saline were injected intra-arterially 5 min before the start of the infusion of L-5-HTP. A second dose was given again 15 min later. The same protocol was followed in all animals so that each animal could serve as its own control. The sequence of experiments was varied and only one experiment per animal was performed each day. During hypoxia the average incidence of FBM per minute was calculated over 5 min intervals.

Statistical methods. Statistical analysis was performed by two-way analysis of variance at each time point. This gives a comparison of each of the three treatments with saline for each animal and is more efficient than separate *t* tests (Snedecor & Cochran, 1980). In order to meet the criteria necessary for analysis of variance, a natural logarithmic transformation was taken of the numbers of breaths during hypoxia. To simplify the figures each treatment is represented by points joined by a continuous line rather than by multiple bar charts.

RESULTS

Experiments in normoxia

At the start of experiments, fetal arterial blood gas and pH values were: P_{a,O_2}, 24·0±0·5 mmHg; P_{a,CO_2}, 47·0±0·7 mmHg; pH_a, 7·330±0·004). Normal episodes of ECoG and FBM occurred. Blood gas and pH values were not altered significantly

Fig. 2. The electrocortical activity (ECoG), diaphragm electromyogram (EMG) and tracheal minus amniotic pressure records for fetus No. 83/56 during infusion of saline on day 132 of gestation (*A*), L-5-HTP on day 121 (*B*), L-5-HTP with ketanserin (Ket) on day 122 (*C*) or L-5-HTP with cyproheptadine (Cypro) on day 124 (*D*).

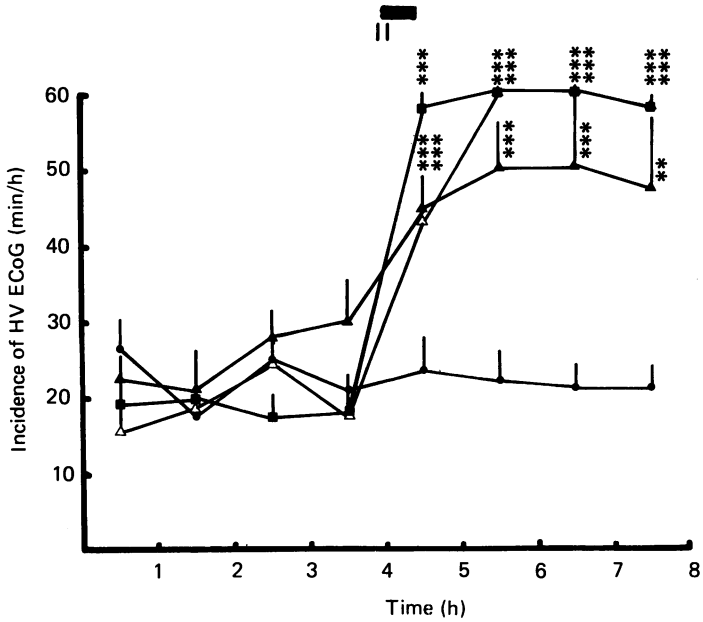

Fig. 4. The effects on mean (+S.E.M.) incidence of HV ECoG over the 8 h period. ▬ indicates when saline or L-5-HTP was infused and || indicates injection of saline, ketanserin or cyproheptadine. ●, ▲, ■, △ indicate the experiments with saline (seven animals), L-5-HTP (seven animals), L-5-HTP with cyproheptadine (six animals) or L-5-HTP with ketanserin (six animals). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ *vs.* saline. The differences between L-5-HTP with cyproheptadine and L-5-HTP with ketanserin were significant at the 5th hour (not indicated).

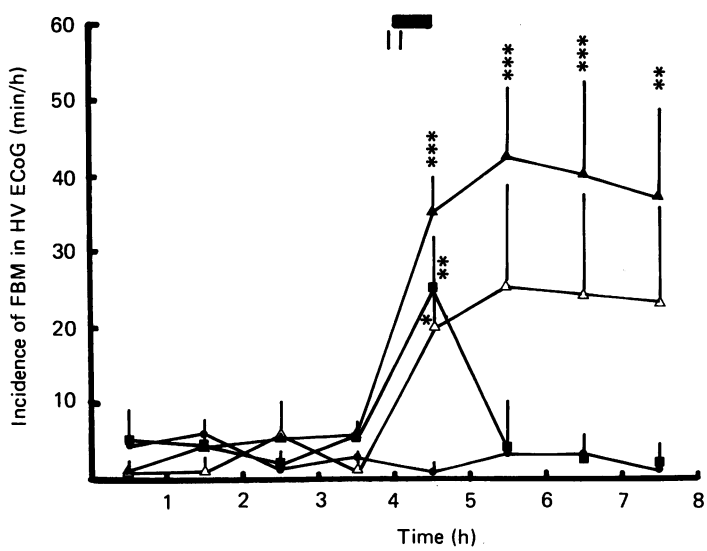

Fig. 5. The effects on mean (+S.E.M.) incidence of FBM in HV EcoG over the 8 h period. ▬ indicates when saline or L-5-HTP was infused and || indicates injection of saline, ketanserin or cyproheptadine. ●, ▲, ■, △ indicate the experiments with saline (seven animals), L-5-HTP (seven animals), L-5-HTP with cyproheptadine (six animals) or L-5-HTP with ketanserin (six animals). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ *vs.* saline. The differences between L-5-HTP and L-5-HTP with cyproheptadine were significant at hours 6, 7 and 8 and those between L-5-HTP with ketanserin were significant at hours 5, 6 and 7 (not indicated).

either during infusion of L-5-HTP alone or when it was given in conjunction with ketanserin or cyproheptadine.

Arterial blood pressure. Figure 1 summarizes the effects of the drugs on mean arterial pressure. There were no significant differences between the groups before infusions began. Infusion of L-5-HTP increased mean blood pressure significantly above the saline value over the next 4 h. Injection of cyproheptadine blocked this increase in pressure. The effect of L-5-HTP was significantly less when ketanserin was

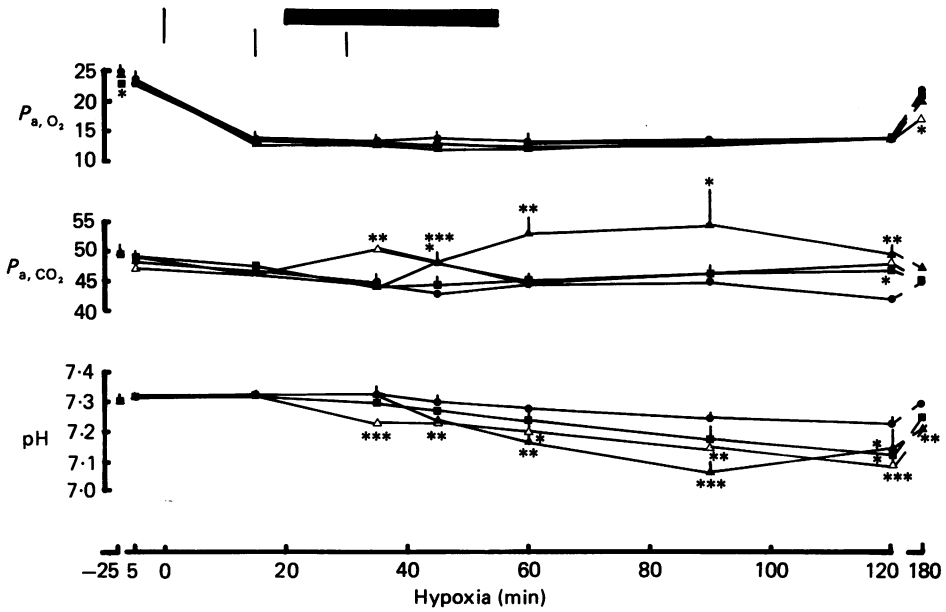

Fig. 6. The effects on mean (+S.E.M.) blood gases and pH during hypoxia. ▬ indicates when saline or L-5-HTP was infused and || indicates injection of saline, ketanserin or cyproheptadine. ●, ▲, ■, △ indicate the experiments with saline (eight animals), L-5-HTP (six animals), L-5-HTP with cyproheptadine (six animals) or L-5-HTP with ketanserin (six animals). $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$ *vs.* saline. (Occasional significant differences between other treatments are not indicated.)

injected but it was still significantly greater when compared to saline. There was no significant difference between the effects of L-5-HTP with cyproheptadine and L-5-HTP with ketanserin.

Breathing and electrocortical activity. Figure 2 shows examples from one animal of the effects on FBM and ECoG and Figs 3, 4 and 5 summarize these results for all animals. Infusion of L-5-HTP increased the incidence of FBM over the next 4 h, this increase being significant in the first 2 h (see Fig. 3). The increase was blocked by ketanserin and cyproheptadine. Cyproheptadine also reduced the incidence of FBM below saline control values and the reduction was significant in the third and fourth hours.

Infusion of L-5-HTP significantly increased the incidence of HV ECoG over the next 4 h (Fig. 4). This increase in HV ECoG was not blocked by ketanserin or cyproheptadine.

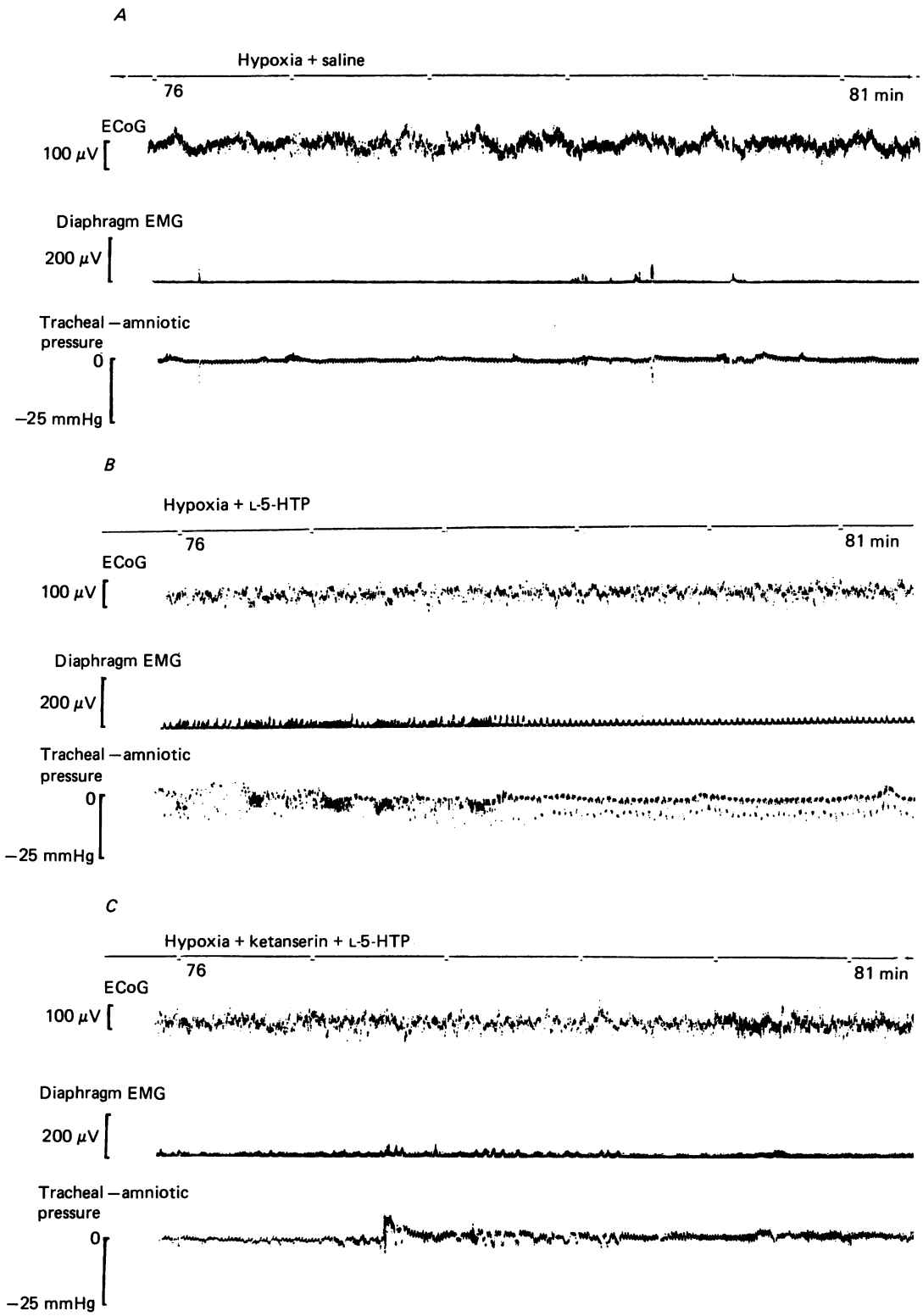

Fig. 7 *A–C*. For legend see opposite.

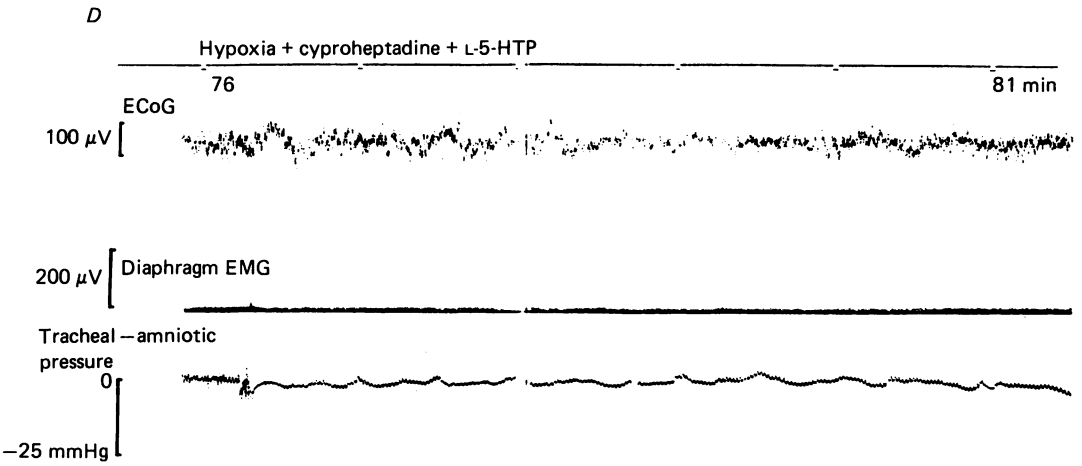

Fig. 7. The electrocortical activity (ECoG), diaphragm electromyogram (EMG) and tracheal minus amniotic pressure records for fetus No. 83/46 over minutes 76–81 of hypoxia after infusion of saline on day 125 of gestation (*A*) and L-5-HTP on day 126 (*B*). L-5-HTP with ketanserin on day 127 (*C*) or L-5-HTP with cyproheptadine on day 128 (*D*).

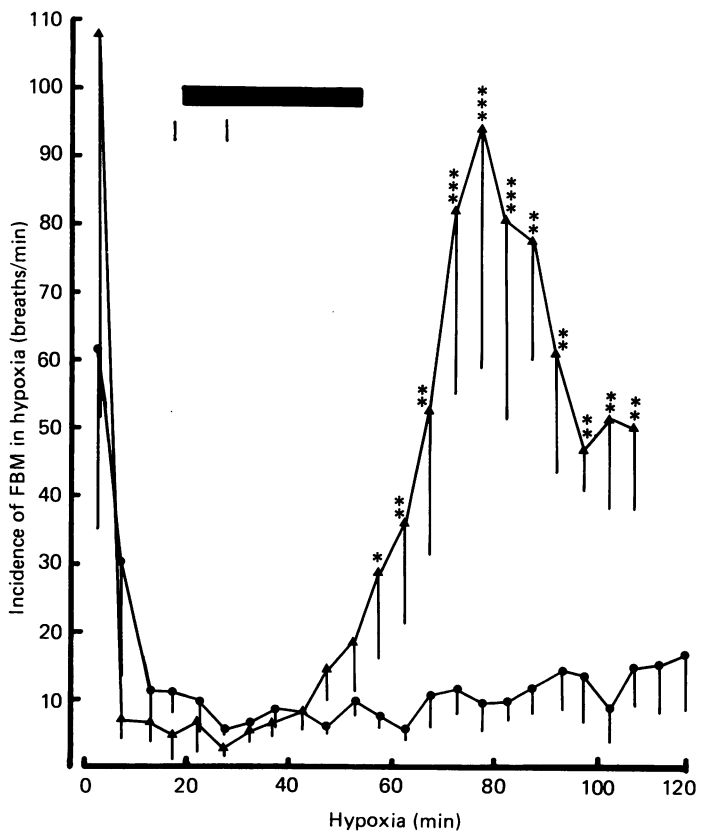

Fig. 8. The effects on mean (−S.E.M.) incidence of FBM during hypoxia. ▬ indicates when saline or L-5-HTP was infused and || indicates injection of saline. ●, ▲ indicate the experiments with saline (eight animals) or L-5-HTP (six animals). $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$ *vs.* saline.

Figure 5 shows that infusion of L-5-HTP significantly increased the incidence of FBM in HV ECoG over the next 4 h. Although ketanserin and cyproheptadine had prevented the effects of L-5-HTP on FBM, there was still significantly more breathing during HV ECoG in the first hour than after saline. Subsequently cyproheptadine reduced the incidence of FBM and therefore of FBM in HV ECoG.

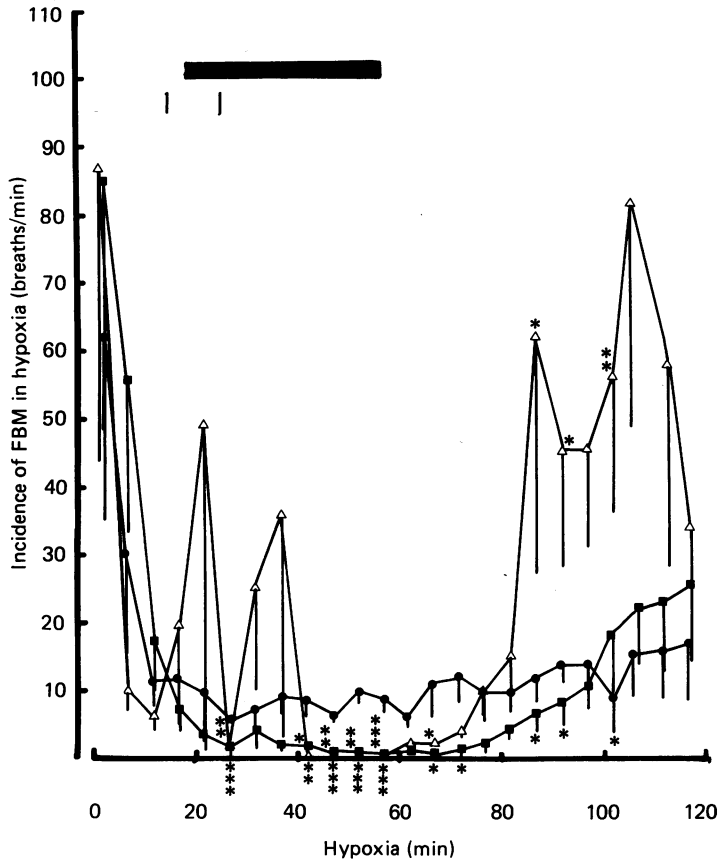

Fig. 9. The effects on mean (−S.E.M.) incidence of FBM during hypoxia. ▬ indicates when saline or L-5-HTP was infused and || indicates injection of saline, ketanserin or cyproheptadine. ●, △, ■ indicate the experiments with saline (eight animals), L-5-HTP with ketanserin (seven animals) or L-5-HTP with cyproheptadine (seven animals). $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$ *vs.* saline. The differences between L-5-HTP and L-5-HTP with cyproheptadine were significant between minutes 50 and 110 and those between L-5-HTP and L-5-HTP with ketanserin were significant between minutes 45 and 85.

Experiments in hypoxia

In hypoxia the mean fetal P_{a,O_2} was reduced by about 11 mmHg (Fig. 6). P_{a,O_2} remained constant throughout the 2 h period and was unaffected by drug treatment. With infusion of saline, P_{a,CO_2} and pH_a fell slightly over the 2 h period. With L-5-HTP, the changes in pH were greater, and P_{a,CO_2} rose, the differences from saline being significant starting from the twentieth minute after the onset of the infusion of L-5-HTP. One animal died 95 min after this infusion began. In the presence of

either antagonist the changes in pH and P_{a,CO_2} were generally smaller than for L-5-HTP alone and these changes were also less consistent (see Fig. 6).

Figure 7 illustrates the effects of the various infusions in one animal (76–81 min after the onset of hypoxia) and Figs 8 and 9 summarize the results in all animals over the 2 h period. In the saline-infusion experiment hypoxia greatly reduced (but did not abolish) the incidence of FBM over the 2 h period (see Fig. 8). Infusion of L-5-HTP increased the incidence of FBM during hypoxia. The increase was significant from 40 min after infusion began and the maximum effect occurred 60 min later. The FBM during hypoxia was in general of normal amplitude (see Fig. 7). Cyproheptadine blocked the increase in the incidence of FBM induced by L-5-HTP, reducing the incidence below that during saline (see Fig. 9). Ketanserin delayed the increase in FBM for 35 min but thereafter it did not significantly reduce the effects of L-5-HTP.

DISCUSSION

Effects in normoxia

Quilligan *et al.* (1981) reported that in five fetuses aged 127–131 days infusion of L-5-HTP induced at least 2·5 h of continuous HV ECoG and FBM, but that in four fetuses aged 116–129 days L-5-HTP did not stimulate FBM. Johnston, Walker & Green (1984), however, tried unsuccessfully to extend these observations by attempting to deplete central 5-HT synthesis and hence to alter FBM. We have confirmed that L-5-HTP alone stimulates FBM, but here the stimulation was seen in all experiments, the earliest being at 122 days gestation. In addition we have demonstrated that the effect can be blocked by cyproheptadine and ketanserin.

Like Quilligan *et al.*, we observed a prolonged period of HV ECoG after infusion of L-5-HTP. During this HV ECoG continuous FBM occurred, a combination not normally seen in fetal sheep. Bamford, Dawes, Parkes & Quail (1984) reported that atropine also produces a prolonged episode of HV ECoG with FBM occurring, although in this case FBM remained episodic. Both drugs therefore uncouple the normal link between the ECoG state and the occurrence of FBM. This suggests that these two processes originate independently and opposes the idea (e.g. Koos, Sameshima & Power, 1987) that HV ECoG itself prevents the occurrence of FBM.

When analysing the mechanisms whereby 5-HT antagonists block the effects of L-5-HTP, the reports that all 5-HT antagonists show some affinity for other receptors must be considered. Antagonism of dopaminergic mechanisms may be excluded since the binding of ketanserin and cyproheptadine to dopamine receptors *in vitro* is not paralleled by the antagonism of dopamine agonists *in vivo* (Leysen *et al.* 1981; Awouters, 1985). Antagonism of muscarinic or adrenergic mechanisms may be excluded where ketanserin and cyproheptadine have similar effects, since only cyproheptadine antagonizes muscarinic activity and only ketanserin antagonizes adrenergic activity (Leysen *et al.* 1981; Awouters, 1985). It is possible that these antagonists bind to histamine receptors in the fetal CNS as well as to 5-HT receptors and that this blocking of histamine receptors prevented the stimulation of FBM by L-5-HTP. This explanation seems unlikely, however, as there is no precedent to indicate that central histamine receptors control fetal or post-natal breathing (Bryan, Bowes & Maloney, 1986; Dempsey *et al.* 1986).

There remains the proposal that effects of L-5-HTP which are blocked by 5-HT antagonists are mediated via 5-HT receptors. Post-natally three subtypes of 5-HT receptor have been described, 5-HT_1 and 5-HT_2 receptors being in the peripheral and central nervous systems and 5-HT_3 in only the peripheral nervous sytem (Bradley, Engel, Fenuik, Fozard, Humphrey, Middlemiss, Mylecharane, Richardson & Saxena, 1986; Bradley, 1987). No antagonist has been found to bind all three types of receptor. Post-natally ketanserin and cyproheptadine bind to 5-HT_2 receptors while cyproheptadine also binds with lower affinity to 5-HT_1 receptors; neither drug binds to 5-HT_3 receptors (Leysen *et al.* 1981; Bradley *et al.* 1986). The classification of 5-HT receptor subtypes has not yet been undertaken in the fetus.

Since ketanserin and cyproheptadine have qualitatively similar effects on FBM in normoxia it is appropriate to propose that their common actions are via the same receptor subtype. If the post-natal classification of 5-HT subtype is appropriate to the fetus, our results indicate that the increase in the incidence of FBM caused by L-5-HTP is mediated predominantly via 5-HT_2 receptors. Cyproheptadine, however, reduced the incidence further than did ketanserin. Since cyproheptadine has a lower affinity than ketanserin for 5-HT_2 receptors and only half as much as of ketanserin was given, our findings suggest that 5 HT_1 receptors are also involved in the stimulation of FBM by L-5-HTP. Moreover, since the incidence of FBM after cyproheptadine was less than that after saline, 5-HT_1 receptors might also have a role in the regulation of normal FBM. Testing these ideas necessitates the use of more specific 5-HT_1 antagonists than are presently available.

Neither ketanserin nor cyproheptadine prevented the induction of HV ECoG by L-5-HTP. The HV ECoG state in the fetus induced by L-5-HTP therefore appears to be different from the slow-wave sleep state induced by L-5-HTP in the post-natal animal, where the effects are blocked by 5-HT antagonists (Sallanon, Buda, Janin & Jouvet, 1982). There may be several reasons for this difference. First, effects of L-5-HTP which are not blocked by 5-HT antagonists may not involve 5-HT receptors. For example, while 5-HT metabolites are not converted to catecholamines, being excreted with the indole ring intact, they can compete with catecholamines for enzymes such as monoamine oxidases (Cooper *et al.* 1982) and might upset the balance of catecholamine metabolism in the fetus. Alternatively a novel type of 5-HT receptor might be involved in regulation in the fetal brain. A final consideration is that the HV ECoG state in the fetus may not be analogous to the slow-wave electrocortical activity seen during sleep post-natally, since the fetus is at an earlier stage of development and under different physical conditions.

Effects in hypoxia

We have demonstrated that infusion of L-5-HTP during hypoxia increases the incidence of FBM. This is similar to its stimulatory effects in normoxia. In addition this increase is blocked by cyproheptadine and delayed by ketanserin. In hypoxia L-5-HTP also induced an increase in P_{a,CO_2} about 15 min before the onset of FBM and a decrease in pH_a at about the same time. For several reasons it is unlikely that these changes induced the onset of FBM. First, FBM are known to be stimulated almost immediately by a rise in P_{a,CO_2} (Boddy *et al.* 1974) or, only after several hours, by a fall in pH (Molteni, Melmed, Sheldon, Jones & Meschia, 1980). Moreover, when

hypoxia and hypercapnia are combined, the inhibitory effects of hypoxia overcome the stimulatory effects of hypercapnia (Dawes *et al.* 1982; Johnston & Walker, 1986). Secondly, similar changes occurred when L-5-HTP was infused with ketanserin, yet FBM were not significantly stimulated when these changes occurred.

Cyproheptadine was equally effective in blocking the effects of L-5-HTP in normoxia and hypoxia. Ketanserin however was less potent in hypoxia. Thus in hypoxia the stimulation of FBM by L-5-HTP may be predominantly via 5-HT_1 receptors.

Knowledge of the drugs which restart FBM during isocapnic hypoxia may reveal the mechanism underlying the inhibition of FBM by hypoxia. As well as L-5-HTP, FBM can be initiated during hypoxia by the dopamine agonist apomorphine (Bamford, Dawes & Ward, 1986) and the muscarinic agonist pilocarpine (Hanson *et al.* 1987) and the peptide thyroid releasing hormone (Bennet, Gluckman & Johnston, 1988). Alternatively, a number of drugs have been shown not to restart FBM during isocapnic hypoxia; these include the peripheral chemoreceptor stimulant doxapram (Bamford, Dawes, Hanson & Ward, 1986), the prostaglandin synthetase inhibitor meclofenamate (Koos, 1985), the opiate antagonist naloxone (Adamson, Patrick & Challis, 1984), the GABA antagonist picrotoxin (Johnston & Gluckman, 1983), the β-adrenergic agonist isoprenaline (Jansen, Ioffe & Chernick, 1986) and the α_2-adrenergic agonist idazoxan (Bamford, Dawes, Denny & Ward, 1986). While the list is not exhaustive it is striking that, to date, only drugs classified as receptor agonists restart FBM during hypoxia. We do not yet know whether this indicates that the lack of FBM in hypoxia is due to a paucity of the appropriate neurotransmitters (e.g. acetylcholine, 5-HT or dopamine) or simply that excess neurotransmitters can overcome the inhibition of FBM by hypoxia.

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REFERENCES

ADAMSON, S. L., PATRICK, J. E. & CHALLIS, J. R. G. (1984). Effects of naloxone on the breathing, electrocortical, heart rate, glucose and cortisol responses to hypoxia in the sheep fetus. *Journal of Developmental Physiology* **6**, 495–507.

AWOUTERS, F. (1985). The pharmacology of ketanserin, the first selective serotonin S_2 antagonist. *Janssen Pharmaceutica Research Laboratories Report* N43898.

AXELROD, J. & INSCOE, J. K. (1963). Uptake and binding of circulating serotonin and the effect of drugs. *Journal of Pharmacology and Experimental Therapeutics* **141**, 161–165.

BAMFORD, O. S., DAWES, G. S., DENNY, R. & WARD, R. A. (1986). Effects of the α_2-adrenergic agonist clonidine and its antagonist idazoxan on the fetal lamb. *Journal of Physiology* **381**, 29–37.

BAMFORD, O. S., DAWES, G. S., HANSON, M. A. & WARD, R. A. (1986). The effects of doxapram on breathing, heart rate and blood pressure in fetal lambs. *Respiration Physiology* **66**, 387–396.

BAMFORD, O. S., DAWES, G. S., PARKES, M. J. & QUAIL, A. W. (1984). Atropine, electrocortical activity and breathing in fetal lambs. *Journal of Physiology* **349**, 59*P*.

BAMFORD,O. S., DAWES, G. S. & WARD, R. A. (1986). Effects of apomorphine and haloperidol in fetal lambs. *Journal of Physiology* **377**, 37–47.

BENNET, L., GLUCKMAN, P. D. & JOHNSTON, B. M. (1988). The central effects of thyrotropin-releasing hormone on the breathing movements and electrocortical activity of the fetal sheep. *Pediatric Research* **23**, 72–75.

BODDY, K., DAWES, G. S., FISHER, R., PINTER, S. & ROBINSON, J. S. (1974). Fetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. *Journal of Physiology* **243**, 599–618.

BRADLEY, P. B. (1987). 5-HT3 receptors in the brain? *Nature* **330**, 696.

BRADLEY, P. B., ENGEL, G., FENUIK, W., FOZARD, J. R., HUMPHREY, P. P. A., MIDDLEMISS, D. N., MYLECHARANE, E. J., RICHARDSON, B. P. & SAXENA, P. R. (1986). Proposals for the classification and nomenclature of functional receptors for 5 hydroxytryptamine. *Neuropharmacology* **25**, 563–576.

BRYAN, A. C., BOWES, G. & MALONEY, J. E. (1986). Control of breathing in the fetus and newborn. In *Handbook of Physiology*, section 3, *The Respiratory System*, vol. II, part 1, pp. 621–647. Bethesda, MD, U.S.A.: American Physiological Society.

CLEWLOW, F., DAWES, G. S., JOHNSTON, B. M. & WALKER, D. W. (1983). Changes in breathing, electrocortical and muscle activity in unanaesthetized fetal lambs with age. *Journal of Physiology* **341**, 463–476.

COOPER, J. R., BLOOM, F. E. & ROTH, R. H. (1982). *The Biochemical Basis of Neuropharmacology*. Oxford: Oxford University Press.

DAWES, G. S., FOX, H. E., LEDUC, B. M., LIGGINS, G. C. & RICHARDS, R. T. (1972). Respiratory movements and rapid eye movement sleep in the fetal lamb. *Journal of Physiology* **220**, 119–143.

DAWES, G. S., GARDNER, W. G., JOHNSTON, B. M. & WALKER, D. W. (1982). Effects of hypercapnia on tracheal pressure, diaphragm and intercostal electromyograms in unanaesthetized fetal lambs. *Journal of Physiology* **326**, 461–474.

DAWES, G. S., GARDNER, W. G., JOHNSTON, B. M. & WALKER, D. W. (1983). Breathing in fetal lambs: effects of brain stem section. *Journal of Physiology* **335**, 535–553.

DEMPSEY, J. A., OLSON, E. B. & SKATRUD, J. B. (1986). Hormones and neurochemicals in the regulation of breathing. In *Handbook of Physiology*, section 3, *The Respiratory System*, vol. II, part 1, pp. 181–221. Bethesda, MD, U.S.A.: American Physiological Society.

GLUCKMAN, P. D. & JOHNSTON, B. M. (1987). Lesions in the upper lateral pons abolish the hypoxic depression of breathing in unanaesthetized fetal lambs *in utero*. *Journal of Physiology* **382**, 373–383.

GOODMAN GILMAN, A., GOODMAN, L. S., RALL, T. W. & MURAD, F. (1985). *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. New York: Macmillan Publishing Company.

HANSON, M. A., MOORE, P. J., NIJHUIS, J. N. & PARKES, M. J. (1987). Stimulation of fetal breathing movements during hypoxia by L-5-hydroxytryptophan in sheep. *Journal of Physiology* **394**, 169*P*.

JANSEN, A. H., IOFFE, S. & CHERNICK, V. (1986). Stimulation of fetal breathing activity by β-adrenergic mechanisms. *Journal of Applied Physiology* **60**, 1938–1945.

JOHNSTON, B. M. & GLUCKMAN, P. D. (1983). GABA mediated inhibition of breathing in the late gestation sheep fetus. *Journal of Developmental Physiology* **5**, 353–360.

JOHNSTON, B. M. & WALKER, D. W. (1986). Respiratory responses to changes in oxygen and carbon dioxide in the perinatal period. In *Respiratory Control and Lung Development in the Fetus and Newborn*, ed. JOHNSTON, B. M. & GLUCKMAN, P. D., pp. 280–303. New York: Perinatology Press.

JOHNSTON, B. M., WALKER, D. W. & GREEN, A. R. (1984). Lack of long-term effect of p. chlorophenylalanine on brain 5-HT and electrocortical activity in conscious fetal sheep. *Experientia* **40**, 308–309.

JOUVET, M. (1972). The role of monoamines and acetyl choline containing neurones in the regulation of the sleep–waking cycle. *Ergebnisse der Physiologie* **64**, 166–367.

KOELLA, W. P. (1984). The organisation and regulation of sleep. *Experientia* **40**, 309–408.

KOOS, B. (1985). Central stimulation of breathing movements in fetal lambs by prostaglandin synthetase inhibitors. *Journal of Physiology* **362**, 455–466.

KOOS, B., SAMESHIMA, H. & POWER, G. G. (1987). Fetal breathing, sleep state and cardiovascular responses to graded hypoxia in sheep. *Journal of Applied Physiology* **62**, 1033–1039.

LEYSEN, J. E., AWOUTERS, F., KENNIS, L., LADURON, P. M., VANDENBECK, J. & JANSSEN, P. A. J. (1981). Receptor binding profile of R 41 486, a novel antagonist at 5-HT receptors. *Life Sciences* **28**, 1015–1022.

MOLTENI, R. A., MELMED, M. H., SHELDON, R. E., JONES, M. D. & MESCHIA, G. (1980). Induction

of fetal breathing by metabolic acidemia and its effects on blood flow to the respiratory muscles. *American Journal of Obstetrics and Gynecology* **136**, 609–615.

QUILLIGAN, E. J., CLEWLOW, F., JOHNSTON, B. M. & WALKER, D. W. (1981). Effect of 5-hydroxytryptophan on electrocortical activity and breathing movements of fetal sheep. *American Journal of Obstetrics and Gynecology* **141**, 271–275.

SALLANON, M., BUDA, C., JANIN, M. & JOUVET, M. (1982). 5 Hydroxytryptamine antagonists suppress sleep and delay its restoration after 5 hydroxytryptophan in parachlorophenylalanine pretreated cats. *European Journal of Physiology* **82**, 29–35.

SNEDECOR, G. W. & COCHRAN, W. G. (1980). *Statistical Methods*. Iowa: Iowa State University Press.

STONE, C. A., WANGER, H. C., LUDDEN, C. T., STAVORSKI, J. M. & ROSS, C. A. (1961). Antiserotonin-antihistaminic properties of cyproheptadine. *Journal of Pharmacology and Experimental Therapeutics* **131**, 73–84.